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Use of Self-Collected Capillary Blood Samples for Islet Autoantibody Screening in Relatives: A Feasibility and Acceptability Study

Running head: Islet autoantibody screening through self-collected capillary blood sampling

Y. Liu[1], L.E. Rafkin[2], D. Matheson[2], C. Henderson[3], D. Boulware[3], R.E.J. Besser[4], C. Ferrara[5], L. Yu[6], A.K. Steck[6], P.J. Bingley[7] and the Type 1 Diabetes TrialNet Study Group*.

1. Division of Diabetes and Nutritional Sciences, King's College London, UK
2. University of Miami Miller School of Medicine, USA
3. University of South Florida Health Informatics Institute, USA
4. John Radcliffe Hospital, Oxford, UK
5. Division of Pediatric Endocrinology and Diabetes, University of California, San Francisco, USA
6. Barbara Davis Center for Childhood Diabetes, University of Colorado School of Medicine, Aurora, CO, USA
7. School of Clinical Sciences, University of Bristol, UK

* A complete list of the Type 1 Diabetes TrialNet Study Group is included in the supplemental material.

Corresponding author: Polly J Bingley

Professor of Diabetes, School of Clinical Sciences, Faculty of Health Sciences, University of Bristol Email: polly.bingley@bristol.ac.uk

Tel: +44 (0) 117 414 8034 Fax: +44 (0) 117 414 8069

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Novelty statement (bulleted, up to 100 words)

- This is the first study to evaluate use of capillary blood samples, collected at home by families themselves, for islet autoantibody testing.
- Capillary sampling was feasible and acceptable in all age groups including young children, with high rates of success in testing for GAD, IA2 and ZnT8 autoantibodies.
- The study highlights that insulin autoantibody assays were least likely to be successful, and therefore to avoid missing very young children at risk, second line venous sampling may be required in this group.
- Screening of islet autoantibodies using this method is popular with families of people with type 1 diabetes, particularly children.

Abstract

Aims: To evaluate the feasibility of using self-collected capillary blood samples for islet autoantibody testing to identify risk in relatives of people with type 1 diabetes.

Methods: Participants were recruited via the observational TrialNet Pathway to Prevention study, which screens and monitors relatives of people with type 1 diabetes for islet autoantibodies. Relatives were sent kits for capillary blood collection, with written instructions, an online instructional video link and a questionnaire. Sera from capillary blood samples were tested for autoantibodies to glutamic acid decarboxylase (GADA), islet antigen-2 (IA-2A), insulin (IAA), and zinc transporter 8 (ZnT8A). 'Successful' collection was defined as obtaining sufficient volume and quality to provide definitive autoantibody results, including confirmation of positive results by repeat assay.

Results: In 240 relatives who returned samples, the median age was 15.5 years (range 1-49) and 51% were male. **Of these samples, 98% were sufficient for GADA/IA-2A/ZnT8A, and 84% for IAA testing and complete autoantibody screen.** The upper 90% confidence bound for unsuccessful collection was 4.4% for GADA, IA-2A and/or ZnT8A assays, and 19.3% for IAA. Despite 43% of 220 questionnaire respondents finding capillary blood collection uncomfortable or painful, 82% preferred home self-collection of capillary blood samples compared with outpatient venepuncture (90% <8 years, 83% 9-8 years and 73% >18 years). The perceived difficulty of collecting capillary blood samples did not affect success rate.

Conclusions: Self-collected capillary blood sampling offers a feasible alternative to venous sampling, with the potential to facilitate autoantibody screening for type 1 diabetes risk.

Introduction

Islet autoantibodies are indicators of β-cell autoimmunity and individuals with two or more islet autoantibodies have >80% risk of developing type 1 diabetes within 15 years[1]. A recent type 1 diabetes staging classification system emphasised the importance of identifying islet autoimmunity in early pre-symptomatic stages, potentially allowing for earlier intervention[2]. Prevention of progression to symptomatic disease requires large-scale screening to identify individuals at risk. Barriers to screening include geographical, cost and time constraints, as well as aversion to venepuncture, particular in children. A simple test allowing self-collection of samples at home could overcome these limitations. **Capillary sampling**, having previously been validated against venous sampling for detection of islet autoantibodies[3–5], offers a potential solution. We assessed feasibility and acceptability of home self-collection of **capillary samples** for autoantibody screening in relatives of people with type 1 diabetes.

Methods

The TrialNet Pathway to Prevention (PTP) study[6] recruited relatives from 15 US centres between August and December 2015. **Institutional review boards at each centre provided ethical approval. Families provided written informed consent. Some centres offered telephone consultations with postal return of consent forms. Samples** were tested for GADA, IA-2A, IAA, and ZnT8A in accordance with the PTP study protocol **replacing venous sampling.** Participants positive for ≥1 autoantibody or with unsuccessful

capillary sampling were recalled for confirmatory venous testing. **Age-banded** recruitment ensured enrolment of adequate numbers of younger children.

Capillary sampling kits, containing BD Microtainer® contact-activated lancets (Becton Dickinson, Franklin Lakes, NJ), Sarstedt Microvette® serum gel capillary tubes with clotting activator (Sarstedt Inc., Newton, NC) **with written and online instructions were provided in person or by post**. A minimum volume of 200µl (up to 500µl) was requested. Adults performed the procedure on children <12 years, whilst children aged 12-17 **collected samples** themselves, or aided by an adult. Acceptability of **collection** was assessed by questionnaire (supplementary materials). Samples were returned to clinical sites **by overnight courier**. Extracted serum was stored and sent to the central laboratory at -20^{o}C.

GADA, IA-2A, ZnT8A, and IAA in capillary serum samples were measured by radioimmunoassay in the TrialNet Core laboratory at the Barbara Davis Center for Childhood Diabetes as **previously described [6].** The same autoantibody cut-offs were used for capillary serum as used for venous serum in TrialNet studies.

The primary outcome of the study was successful **sample** collection, defined as sufficient volume and quality (e.g. without excessive haemolysis) to allow definitive autoantibody results, including confirmation of positive results by repeat assay. Secondary outcomes incorporated acceptability of **sample**

collection and additional analyses to formulate an upper confidence bound of unsuccessful **sampling**.

Results are presented as median (range) unless otherwise stated. The upper confidence bound of unsuccessful **sampling** was calculated using the Clopper-Pearson interval. Chi-squared tests were performed **for categorical values**. Non-parametric questionnaire scoring data were compared using Wilcoxon rank sum and Kruskal-Wallis testing. Logistic regression was used to test for age effects in the risk of unsuccessful **sample** collection. P-values <0.05 were considered significant. Statistical analysis was performed using SAS (SAS, Inc.) and S-PLUS (TIBCO Software Inc.) software.

Results

Table 1 summarises the demographic characteristics of **participants.** The **median** interval between initial shipment and sample collection was 8 days (0-104) (n=158) and from sample collection to receipt at clinical centre was 2 days (0-7) (n=169).

Rates of successful sample collection varied by autoantibody type (Table 1) with highest success for GADA, IA-2A and ZnT8A screening**. There was no significant haemolysis in any samples.**

Upper 90% confidence bounds of unsuccessful **capillary sample** collection were 4.4% for GADA, IA-2A and ZnT8A assays combined, and 19.3% for IAA. **Sampling was** unsuccessful for ≥1 autoantibody assay in 16.0% of those aged

≤8, 17.5% aged 9-18 and 14.5% aged >18 years. There was no difference in rate of unsuccessful sampling between age groups or overall age effect (p=0.73).

Of five participants with positive autoantibodies, four had confirmatory venous testing. Three showed fully concordant venous and capillary results and one was concordant for GADA and IA-2A positivity but an IAA positive capillary sample was negative on venous sampling. Of 39 individuals with unsuccessful capillary sampling six have provided venous samples to date. All capillary samples were insufficient for IAA only and in subsequent venous samples one individual was IAA positive and 5 were IAA negative.

Capillary collection was considered uncomfortable or painful by 43%. Nonetheless, 82% preferred home capillary **sampling** over outpatient venepuncture. Preference for capillary sampling varied by age; 90% ≤8 years, 83% of 9-18 year-olds and 73% of >18 year-olds, with greater preference among younger children (p=0.01). Median score for ease of testing using a scale from 1 (easy) to 7 (difficult), was 3 (interquartile range: 2-5) with no differences between age groups: 3 (2-5) ≤8 years, 3 (1-4) in 9-18 year-olds and 2 (2-5) >18 years (p=0.39), nor between respondents with successful, compared with unsuccessful, sample collection (p=0.10).

Written instructions were reported as easily understandable by 83.2%. Only 53.6% watched the instructional video, with rate of successful sampling being

76.9% in those who watched versus 85.9% in those who did not (p=0.09). There was no difference in reported difficulty of the procedure between those who watched the video, median score 3 (2-4) and those who did not, 2 (2-5) (p=0.10).

Discussion

Ease of sample collection has allowed widespread adoption of **capillary sampling** in commercial self-testing for conditions such as hypercholesterolaemia and coeliac disease**. Collection by clinicians has been used successfully for large-scale islet autoantibody testing in children[9] including those as young as 2 years**[10,11]**.** However no published data exist on self-collection of **capillary samples** for islet autoantibody testing outside the clinical setting.

Capillary samples have shown high concordance with venous samples when compared for GADA[3–5], IA-2A[3–5] **(or combined GADA/IA-2A assays)[3,4]**, ZnT8A[5] and IAA[4]. Using a panel of GADA, IA-2A and ZnT8A assays, we have previously shown that 95.5% of individuals who were multiple autoantibody positive in venous serum were concordant in capillary samples collected as **dried blood spots** and 98.6% of those who were autoantibody negative were concordant in **dried blood spot** samples[5].

Options for self-collection of capillary samples are direct collection of whole blood into tubes or **dried blood spots** on filter paper, with extraction of serum or eluates respectively. Both techniques offer sufficient stability to allow samples to

be shipped at ambient temperature. Antibody levels are lower in **dried blood** eluates than in venous serum, particularly IAA, and weakly positive autoantibodies **may be missed**[3] introducing a risk of overlooking individuals with a single positive autoantibody. Our previous study which did not include IAA in the initial screen, showed 39% of relatives who were single autoantibody positive in a venous sample were missed by **dried blood spot** sampling[5]. **Assay optimisation on dried blood spots has improved detection of IAA[12], however collection of dried capillary samples, even by clinicians, resulted in variable quality with 45% of samples insufficient to allow confirmation of positive results[5]**.This limits adoption of **dried blood spots** in screening strategies, but collecting capillary whole blood into tubes could overcome these issues.

Of self-collected capillary samples, 16% were suboptimal for testing for all four autoantibodies, due to insufficient volumes for IAA measurement, **whereas** only 3% were insufficient to measure GADA, IA-2A and ZnT8A. The confidence bounds for unsuccessful sample collection for GADA, IA-2A and ZnT8A testing imply **a** 90% certainty that fewer than 4% would need retesting. Incorporating IAA potentially increases **the rate of** unsuccessful **capillary sampling** to 20%, largely because the more complex IAA radioimmunoassay requires five-fold greater volume than the other assays. Relatives were largely successful in collecting sufficient sample volumes to allow GADA, IA-2A and ZnT8A testing. Previously we demonstrated that this panel identified multiple autoantibody positive individuals with high sensitivity[5], the potential risk would therefore be missing individuals who are positive for IAA alone. A fail-safe **capillary** screening strategy would incorporate recall for venous sampling to confirm

positive results and for otherwise autoantibody negative individuals with insufficient sample for IAA testing. Requesting a higher minimum volume could more consistently provide sufficient volumes for all assays. Seroconversion for IAA tends to occur at an early age[13], **which with current assays,** may necessitate continued venous sampling to screen very young children.

Strong preferences were demonstrated for home **capillary sampling** over venepuncture at a clinical centre particularly **among** families of children below 8 years of age. **Extending procedures for obtaining informed consent remotely by telephone or on-line could further facilitate testing.** Our sampling kits appear user-friendly and clear written instructions were adequate for the majority of testers, with few referring to video instructions. **No data were collected from families who declined capillary sampling or unreturned samples, potentially leading to bias.** Importantly, these families were already familiar with capillary glucose testing; **therefore feasibility of capillary sampling in the general population requires further study.**

Self-collected **capillary blood samples** are feasible and acceptable for autoantibody screening in individuals at risk of type 1 diabetes, showing advantages over **dried blood collection** and preferred over outpatient venepuncture, particularly in children. With additional benefits of improving convenience and efficiency, home autoantibody testing through **capillary sampling** could aid type 1 diabetes screening initiatives.

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Table 1: Demographics and rates of successful capillary blood sampling, overall and by age group (n=240). **Successful capillary blood sampling was defined as obtaining samples of sufficient volume and quality (e.g. without excessive haemolysis) to allow definitive autoantibody results, including confirmation of positive results by repeat assay.**

	Overall	Age (years)		
		<=8 (n=81)	9-18 (n=97)	>18 (n=62)
Age Median (range)	15.5 (1-49)			
Gender n(%) Male Female Missing	 121 (51.5) 114 (48.5) 5	 39 (49.4) 40 (50.6) 2	 56 (57.7) 41 (42.3) 0	 26 (44.1) 33 (55.9) 3
Overall successful sample rate n (%)	201 (83.8)	68 (84.0)	80 (82.5)	53 (85.5)
GADA/ZnT8A/IA-2A successful sample rate n (%)	234 (97.5)	80 (98.8)	93 (95.9)	61 (98.4)
IAA successful sample rate n (%)	202 (84.2)	68 (84.0)	81 (83.5)	53 (85.5)